VIRGINIA AGRICULTURAL COUNCIL PREPROPOSAL FORM

Title: Identification of Genes Conferring Drought and Flooding Tolerance in Soybean through Virus-Induced Gene Silencing

Objectives:

Soybean is a major crop in Virginia. In 2013, soybean was planted in 600,000 acres and generated more than $286 million. However, the average soybean yield in Virginia (34.3 bu/ac) is lower than the national average (42.2 bu/ac). In addition, variability of soybean yields is large in Virginia (26-42 bu/ac in 2004-2013) as compared to the national average (39.7-43.5 bu/ac in 2004-2013). One of the primary reasons for high variability in soybean yields is **variable precipitation** during the growth season in Virginia. Both **drought** and **flooding/high soil moisture** cause significant reductions in seedling establishment, biomass growth, pollen development, and seed production. To increase and stabilize soybean yields under changing climates in Virginia, it is imperative to improve adaptability to drought and flooding/high soil moisture in the major legume species.

Recent advances in genomic and molecular technology have accelerated the discovery of candidate genes associated with tolerance to environmental stresses including drought and flooding. The next step to developing stress-tolerant varieties is to validate the function of each candidate gene in stress tolerance using genetic modification (GM) technology. However, functional validation of candidate genes by GM-based technology requires at least 2-3 years in soybean. Recently, an efficient and effective validation method for candidate genes, virus-induced gene silencing (VIGS), was developed; this technology needs only 3-4 months to determine whether a candidate gene confers stress tolerance. Using VIGS technology, the functional importance of several candidate genes regulating disease resistance and iron toxicity tolerance has been confirmed in soybean. However, this technology has not been used for drought and flooding tolerance genes in soybean. **The major objective** of this study is to apply VIGS technology for functional validation of candidate genes regulating drought and flooding tolerance in soybean. Development of a new VIGS protocol for drought and flooding tolerance genes will facilitate the creation of new soybean accessions with enhanced stress tolerance.

Approach:

The Fukao Lab has identified nine candidate genes associated with drought and/or flooding tolerance in soybean using genome-scale gene expression analysis technology. To validate the functional importance of these genes in stress tolerance, expression of each candidate gene will be up- or down-regulated in soybean plants using VIGS technology. VIGS consists of three steps; 1) gene cloning, 2) vector construction, and 3) biolistic delivery of vectors into soybean by a gene gun. The Fukao lab has routinely conducted these experiments in rice. Following these steps, up- and down-regulation of each candidate gene in soybean will be confirmed by real-time gene expression analysis. Only confirmed plants will be exposed to drought or flooding tolerance tests to evaluate the effect of transferred genes on stress tolerance. This analysis will allow us to determine whether each candidate gene confers stress tolerance. For example, if up-regulation of Gene A increases drought tolerance and down-regulation decreases tolerance, it is concluded that Gene A is a key regulator for drought tolerance in

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soybean. The degree of stress tolerance will be quantified by whole plant and leaf viability tests, photosynthetic gas exchange assays, and chlorophyll fluorescence measurements. All equipment required for VIGS and stress tolerance evaluation is available in the Fukao lab. The information on gene identity and stress tolerance evaluation obtained from this project will be shared with Dr. Bo Zhang, soybean breeder at Virginia Tech.

Justification:

Soybean growers in Virginia have experienced high yield variability (26-42 bu/ac in 2004-2013) due primarily to variable rainfall during the growth season. It is also anticipated that the number of drought and flooding events will further increase in the U.S. and other countries as a consequence of global climate change. Improvement of soybean adaptability to drought and flooding/high soil moisture will significantly enhance and stabilize the income of soybean growers under changing climates in Virginia. Collaborating with rice breeders, the principal investigator, Takeshi Fukao, has discovered the key gene conferring submergence and drought tolerance in rice and developed new rice cultivars with enhanced stress tolerance. As a new Assistant Professor at Virginia Tech, Fukao is applying his experiences and techniques to identify key regulators of tolerance to drought and flooding in soybean as he conducted in rice.

This project requires various molecular biological supplies (kits, enzymes, chemicals, tubes etc.), which are more expensive than those used for conventional agronomical and physiological analyses. However, the outcome of this project can directly contribute to the enhancement of drought and flooding tolerance in soybean varieties which adapt to Virginia growth conditions. In addition, the VIGS protocol developed in this project will significantly increase the efficiency and effectiveness of functional validation for various stress-tolerance genes in soybean breeding programs in Virginia and other states. Our ultimate goal is to develop and release new soybean varieties with enhanced drought and flooding tolerance in collaboration with Dr. Bo Zhang.

INVESTIGATOR(S)*: Takeshi Fukao (Assistant Professor, Virginia Tech)
DURATION (years) one__x__two_____
BUDGET (yr. one) _$23,000___(total)_$23,000_____
***<u>One</u> pre-proposal per PI please**

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COMMODITY GROUPS	**CHECK *ONE* GROUP THAT BEST DESCRIBES YOUR PROJECT**
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Fruit/Wine	
Livestock Dairy Poultry Hogs Beef Sheep Goats Horses	
Nursery/ Forestry	
Row Crops	X
Turf/Seed	
Vegetable	
Educational	
Miscellaneous Agriculture	